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Delineation of a Duplication Map of Chromosome 3q: A New Case Confirms the Exclusion of 3q25-q26.2 From the Duplication 3q Syndrome Critical Region

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We report on the clinical, cytogenetic, and molecular characterization of a propositus and his mother with a duplication of 3q25-q26, minor anomalies, and mental retardation. The duplication, detected by cytogenetic analysis, was confirmed and delineated by comparative genomic hybridization and fluorescence in situ hybridization using probes previously mapped to the region. Comparison of the mapping data obtained in these patients and those obtained in patients that present with a typical dup(3q) syndrome phenotype shows that the segment duplicated in these patients lies proximally to the reported dup(3q) syndrome critical region, thus explaining the absence in our patients of the characteristic phenotype of dup(3q) syndrome patients. Accumulation of mapping data in patients with segmental duplications of 3q will eventually allow us to build a duplication map of the region and a genotype-phenotype correlation. Am. J. Med. Genet. 68:428–432, 1997.
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KEY WORDS: dup(3q) syndrome; mental retardation; cytogenetic analysis

INTRODUCTION

The dup(3q) syndrome [McKusick MIM number 122470] was first reported as a form of de Lange syndrome with chromosome abnormalities [Falek et al., 1966]. A detailed clinical description was provided by Wilson et al. [1985]. Patients have a characteristic face with hirsutism, synophrys, broad nasal root, anteverted nares, downturned corners of the mouth, and malformed ears. Congenital heart anomalies, in particular septal defects, genitourinary malformations and mental and growth retardation are also common. Cytogenetically, these patients are triploid for a segment of 3q of variable length, usually within the region 3q21-3qter. In a recent report, we proposed the definition of the critical region of the dup(3q) syndrome as delimited by two breakpoints both mapped within 3q26.3-3q27 [Aqua et al., 1995]. A number of publicly available markers have been mapped and ordered in this interval [Rizzu and Baldini, 1994] facilitating the mapping of other patient breakpoints in the region.

The dup(3q) syndrome superficially resembles the Brachmann-de Lange (BDL) syndrome [McKusick MIM number 122470; Jackson et al., 1993]. Wilson et al. [1978] proposed clinical criteria for a distinction between these two syndromes. Intrauterine growth retardation, prominent philtrum, proximally placed thumbs, oligodactyly/phocomelia are more frequent in BDLS, while craniosynostosis, cleft palate, and urinary tract anomalies are more characteristic of the dup(3q) syndrome. BDL patients have apparently normal chromosomes in most cases [review in Opitz, 1985, 1994]. It remains to be resolved whether the underlying biological mechanisms that result in these two syndromes are related.

In this report we present two new cases (mother and son) with a partial duplication of 3q25-3q26. The patients present with a multiple congenital anomaly-mental retardation phenotype that lacks characteristic BDL-like manifestations. Molecular cytogenetic mapping of the duplicated region shows that this is proximal and distinct from the previously reported dup(3q) critical region.

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PATIENTS AND METHODS

Clinical Reports

DB, 8.9 years old, was admitted to hospital with recurrent respiratory infections. Pediatricians referred him to the Genetics Department because of his mental retardation and minor anomalies, which were similar to those of his mother. The propositus is the mother's only child and the father is unknown. He was born at term after an apparently uncomplicated pregnancy with only occasional alcohol ingestion. The delivery was normal, measurements at birth were weight of 2,070 gm, length of 49 cm, and the cranial perimeter was below the 3rd centile (30 cm). The child was diagnosed as having microcephaly with hypotonia (EMG normal) and mild plagiocephaly. His psychomotor development was slightly retarded; he was able to walk at 20 months and began speaking at 24 months. Now at 10.5 years, his height is 139.5 cm (25–50 centile), weight is 45 kg (90–97 centile) and cranial perimeter is 48.5 cm (< 3rd centile). He has mild mental retardation and at school has learning difficulties and is on average 2 years behind his peers. Physical examination shows microcephaly and turribracyhycephaly with slight plagiocephaly. Eyebrows are high arched, the nasal bridge is high, and the nose is long with anteverted nostrils. He has a long philtrum, thin lips, high arched palate, and relative prognathism. The ears are anteverted with hypoplastic helices with irregular borders. He has widely space nipples, brachydactyly with tapered fingers, clinodactyly of 5th fingers and talipes. Radiographies of the hands showed that brachydactyly in this patient is mainly due to distal phalangeal shortness. No joint laxity was noted.

The mother (EB) also has a mild mental retardation; her height is 154 cm (10 centile) and cranial perimeter 48 cm (< 3rd centile). She has microcephaly, high arched eyebrows, upslanting eyes, and high nasal bridge with long and narrow nose. She has hands and fingers similar to those of her son, but the clinodactyly is milder. There are no other relatives with malformations and/or mental retardation.

Cell line GM10266 is derived from a dup(3q) patient (karyotype 46,XY,-22,+der(22)t(3;22)(q26.2;p11)). This patient was described by Falek et al. [1966] as proband 4. The following is a brief clinical summary. The patient was seen at age 14 in an institution for the mentally retarded. Birth weight was 3.7 kg and length was 51 cm. Development was slow and severe retardation became apparent in infancy. He had suffered frequent grand mal seizures since early childhood. At age 14 he had no speech, his height was 141 cm (< 3rd centile), and weight was 41.9 kg (10th centile). His facial features included synophrys, deep set eyes, low forehead, low set ears, pug nose, wide upper lip space, and mild micrognathia. Bilateral nystagmus and internal strabismus were noted. The palate was high and teeth irregular. There was marked hirsutism of the body with irregular patches of baldness. The neck was short; there was a dorsal scoliosis, umbilical hernia, bilateral cryptorchidism, fifth finger clinodactyly, low insertion of thumb, and webbing of the second and third toes.

Fluorescence In Situ Hybridization (FISH) and Molecular Probes

FISH was performed essentially as described in Baldini and Lindsay [1994] on standard chromosome preparations obtained from lymphoblastoid cell lines. At least ten well hybridized metaphase spreads were analyzed per probe. The following cosmid clones were used (in brackets is the cytogenetic assignment): cCI3-607 (3q23), cCI3-581 (3q25.1), cCI3-858 (3q25.3), cCI3-416 (3q26.1-q26.3), cCI3-1366 (3q26.2), cCI3-1350 (3q26.2-q26.3), cCI3-1361(3q26.2), cCI3-1434 (3q26.2), cCI3-413 (3q26.2), cCI3-797 (3q26.31-q27.3) [Takahashi et al., 1992; Yamakawa et al., 1991]. The chromosome 3 painting probe was obtained from Cambio (U.K.).

Comparative Genomic Hybridization (CGH) and analysis of results were performed as described by Haddad et al. [1995]. Briefly, genomic DNA from the patients was labelled with biotin-14-dATP (GIBCO, Grand Island, NY) and control genomic DNA from a healthy male donor was labelled with digoxigenin-11-dUTP (Boehringer, Mannheim, Germany) using standard nick translation procedures. Approximately 400 ng of differentially labelled DNA (200 ng of test DNA and 200 ng of control DNA) was reannealed with 30 μg of Cot-1 DNA (GIBCO, Grand Island, NY) at 37°C for at least 15 minutes and then hybridized at 37°C for 3–4 days to standard chromosome preparations from a normal donor. Biotinylated DNA was detected with FITC-avidin and the digoxigenin labeled DNA with rhodamine anti-digoxigenin antibodies. Chromosomes were counterstained with DAPI (4,6-diamidino-2-phenylindole) for chromosome identification. Digital images of green (test DNA) and red (control DNA), as well as DAPI images for chromosome identification, were collected using a cooled CCD camera (Photometrics CH250 with CCD Kodak KAF1400) mounted on a Zeiss Axioplan epifluorescence microscope and ratio images (green/red) were calculated. Ratio images from 27 chromosomes 3 were subjected to eigenanalysis as described in Haddad et al. [1995] in order to define precisely the ratio variation along the chromosome and an intensity profile was derived from the resulting image. The result of the experimental design is that an increase in ratio value indicates an increase in copy number in the patient DNA relative to the control DNA.

RESULTS AND DISCUSSION

Patients DB and EB are shown in Figure 1 and their clinical findings are summarized in Table I, which also lists the findings of other reported cases. It appears that the phenotype, a multiple congenital anomaly syndrome with mild mental retardation, is not closely related to the "typical" dup(3q) syndrome. In particular, hypertelorism, synophrys, epicanthal folds, downturned mouth, micrognathia, low set ears, and severe mental retardation are absent in DB and EB. Microcephaly, present in our patients, is usually absent in dup(3q). Standard cytogenetic analysis identified the presence of extra material on 3q in DB and EB. To characterize this extra material, we used molecular cytogenetics techniques based on FISH. A chromosome 3 painting probe stained homogeneously both chromosome 3 (not shown), suggesting that the extra material originates from chromo-

Fig. 1. The propositus (DB) and his mother (EB) in a recent photograph.

some 3. Using cosmid clones previously assigned to the region 3q24-q27, we could demonstrate the presence of an interstitial duplication and map the limits of the duplicated segment. FISH results were consistent in all the metaphases studied. CGH analysis confirmed these findings (Fig. 2A–C). Using the same probes we also mapped the position of the duplicated segment of a previously reported patient with typical dup(3q) syndrome. The results are shown schematically in Figure 2D. For comparison, the map also shows the position of the dup(3q) critical region as proposed previously [Aqua et al., 1995]. The region duplicated in DB and EB partially overlaps the region duplicated in GM10266 but is distinct from the dup(3q) critical region. These findings provide an explanation for the absence of the typical dup(3q) phenotype in DB and EB. Lopez-Rangel et al. [1993] have described a patient with a duplication of the region 3q25.1-q26.1 without the characteristic BDLS phenotype. Although no molecular characterization was reported in this case, cytogenetically the duplicated region appears to overlap with that found on DB and EB and the phenotype is consistent with that of our patients, namely, the lack of a typical dup(3q) phenotype. Mild mental retardation and, brachydactyly due to distal phalangeal shortening also appear to be in common. However, microcephaly represents a major difference between our patients and the one previously described.

In summary, the data shown confirm that the BDLS phenotype of the dup(3q) syndrome is determined by the triplication of genes located in a relatively small genomic region in the 3q26.3-q27 region. Furthermore, we show that the duplication of a genomic region proximal to the dup(3q) critical region also generates a multiple congenital anomaly-mental retardation syndrome which is phenotypically distinct from the dup(3q) syndrome. However, this genomic region may contribute to the phenotype in dup(3q) cases which are associated with an extensive duplication including the dup(3q) critical region and other proximal chromosomal bands.

Although DB and EB have apparently the same duplication (at the level of resolution offered by our tests), the phenotype in EB is milder. As in many aneuploidy syndromes, the phenotype may be variable in severity

TABLE I. Main Clinical Findings in Patients With Duplications Within the Region 3q25-qter*

Manifestations	DB	EB	AH	GM10266	SD	KW	Lit. dup 3(q25;qter)[a]
Low birth weight	+	U	ND	−	−	−	+
Hypotonia	+	U	ND	U	+	+	ND
Severe MR	−	−	−	+	+	−	+
Mild MR	+	+	+	−	−	+	−
Abn cranial shape	+	−	−	+	+	−	+
Microcephaly	+	+	−	−	−	−	ND
Synophrys	−	−	−	+	+	−	+
High arched eyebrows	+	+	−	−	+	−	ND
Epicanthal folds	−	−	−	ND	+	+	+
Hypertelorism	−	−	−	ND	+	+	+
High nasal bridge	+	+	−	ND	ND	ND	−
Broad nasal bridge	−	−	−	ND	+	+	+
Anteverted nostrils	+	−	−	ND	+	−	+
Long philtrum	+	−	−	+	ND	ND	+
Down-turned mouth	−	−	−	ND	−	−	+
High arched palate	+	+	ND	+	+	+	+
Micrognathia	−	−	−	+	+	+	+
Low set ears	−	−	−	+	+	−	+
Malformed ears	+	−	+	ND	+	−	+
Brachydactyly	+	+	+	ND	+	ND	+
Tapered fingers	+	+	−	ND	ND	ND	ND
Clinodactyly	+	+	−	+	+	+	+
Simian crease	−	−	ND	ND	+	+	+
Talipes	+	−	−	ND	ND	ND	+

* AH, described in Lopez-Rangel et al., 1993. GM10266, cell line derived from patient 4 described in Falek et al., 1966. SD and KW, unbalanced offsprings of JL220 and JL47, respectively, described in Aqua et al., 1995. U, unknown; ND, not determined.

[a] Anneren and Gustavson, 1984; van Essen et al., 1991.

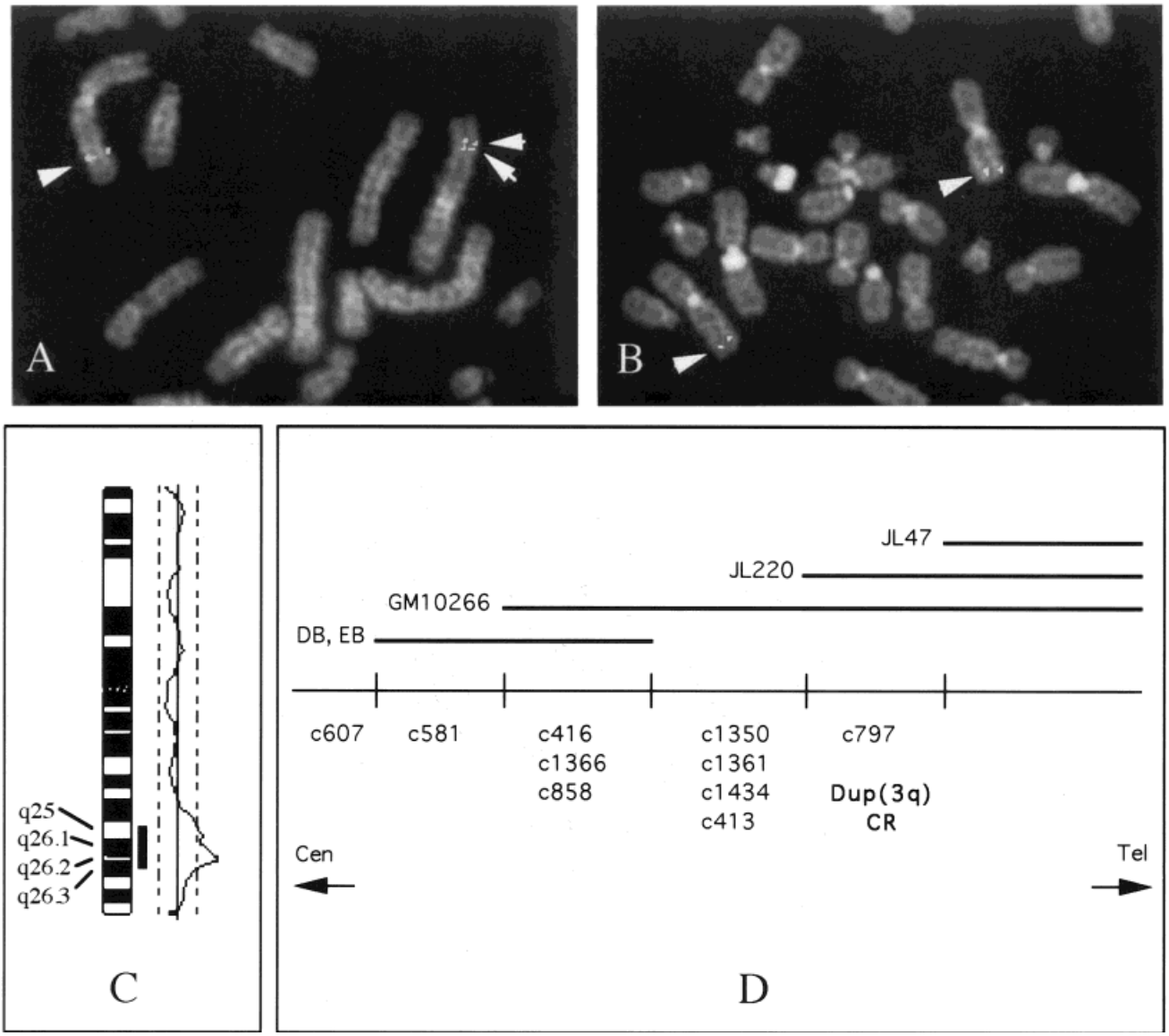

Fig. 2. **A** and **B**: Examples of FISH experiments using chromosome preparations from patient DB. Probe c858 (A) is present in two copies on the duplicated chromosome and c797 (B) is not duplicated. **C**: Ideogram showing the results of CGH experiments. The bar indicates the region found duplicated in DB by this method. **D**: A duplication map of the 3q26-q27 region. Horizontal bars indicate the region found duplicated in the patients indicated. Only GM10266 and JL220 show a dup(3q) syndrome phenotype. On the bottom are indicated the cosmid clones hybridizing to the five intervals defined by patient chromosomal breakpoints and used in the FISH experiments described in this report. The map is not to scale.

even though the chromosomal region involved is the same. Such variability may limit the feasibility of a detailed genotype/phenotype correlation. However, the molecular tools and techniques used in this report along with a framework duplication map should help to further test the hypothesis that duplication of different regions of 3q give rise to distinct phenotypes as new patients are identified.

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